A Comparative Evaluation of Control Measures of *Peronospora plantaginis* Against Isabgol.

Research Protocol

For Registration for Ph.D.Program

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Contents

1. Introduction 4
2. Review of literature 12
3. Statement of the Problem 18
4. The Objective of Study 18
5. Material and Methods 20
6. Significance of Study 22
7. Scope of Study 23
8. Limitation of Study 23
9. References 24

Date:

CERTIFICATE

This is to certify that the Research Proposal entitled "**A Comparative Evaluation of Control Measures of *Peronospora plantaginis* Against Isabgol.**" Submitted for the registration under Ph.D. program by **Arushi Saxena**, In Department of Botany, Faculty of Sciences of JECRC University, Jaipur, in the academic year 2021, is a bonafide record of work carried under my guidance and supervision.

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ABSTRACT:

Plantago ovata Forskaal (Syn. *P.isphagula* Roxb.) is one of the most important cultivated medicinal plants of India. India occupies a 2.65 lakh ha area and contributes about 30% of total Isabgol production. Isabgol faces a lot of fungal disease and bacterial diseases apart from which Downy mildew is the major disease of all. Many fungicidal control measures are known, causing major drawback with respect to harming the environment and biomagnifying the soil infertility. This proposal aims to find out the available biological control Agents like AM Fungi and potential plant parts and plant parts extracts which can be proven as the Bio Control Agent against the downy mildew fungal disease causing *Peronospora plantaginis.*

INTRODUCTION:

India is considered the "Botanical garden of the world" because of its agro-climatic conditions and topography. Many medicinal and aromatic plants constitute about 5000 known species that are used to treat humans as Ayurveda, Unani, and Siddha etc. Medicinal plants are not only a major resource for the ethnic medicine & herbal industries but also provide livelihood and health security to a large segment of the Indian population. About 1178 species of medicinal plants are estimated to be in trade of which 242 species have annual consumption levels over 100 metric tons/year. The domestic demand for medicinal plants has been estimated at 1, 95,000 MT for the year 2014-2015 and the export demand for medicinal plants has been estimated at 1, 34,500 MT during 2014-2015.

Total consumption of the herbal raw drug in the country for the year 2014-15 has been estimated at 5, 12,000 MT with a corresponding trade value of ₹ 5,500 crores. The major increase has been recorded in export value which has increased from ₹ 345.80 Crore in 2005-06 to ₹ 3211 Crore in 2014-15, registering a nine-fold increase during the last decade.

During the 2018-19, season, the overall sowing of Isabgol in the country was nearly 36000 hectares including over 18000 hectares in Rajasthan, nearly 11000 hectares in Madhya Pradesh and around 7300 hectares in Gujarat. Overall production of Psyllium husk was almost 18 lakh bags (of 75kg each) during the season.

Plantago ovata Forskaal (Syn. *P.isphagula* Roxb.) is one of the most important cultivated medicinal plants of India. The Latin name of the crop refers to its appearance of the two parts of the plant – *Plantago* meaning sole of the foot (refers to the leaf shape) and *ovata* meaning ovate shape (refers to the shape of the seed). It is commonly known as blond Psyllium in the international market because of its seed colour.

Classification of *Plantago ovata* according to Engler and Prantl system is as follows:

Kingdom : Plantae

Phylum : Angiospermae

Class : Dicotyledonae

Order : Plantaginales

Family : Plantaginaceae

Genus : *Plantago*

Species : *ovata*

Plantago is an herb with many medicinal qualities which is grown in India and in the USA it also serves as a Laxative which is made from its seed. It is a rabi-season crop generally grown in prone areas of arid and semi-arid regions. It needs a cool and dry climate and generally sown in December. Its flowering begins after the 2 months of sowing and it's harvested in February and March (110 -120 days). It generally stays alive for 130 days due to its short-lived nature. Due to its low cost of production, the area under cultivation of Isabgol is increasing due to its higher returns.

Till 1962, France, Spain, and Brazil were considered to be the principal producer of Isabgol in the world. However, in 1963 Indian Isabgol was recognized as the best in the world and thereafter Indian Isabgol quickly captured the Western market and became the sole supplier of Isabgol to the World market. India occupies a 2.65 lakh ha area and contributes about 30% of total Isabgol production. Isabgol being the most important medicinal plant is cultivated in more than 30933 ha of land in Gujarat with 14067 million tonnes production. It is also known as blond psyllium. *Plantago* belongs *to* the Plantaginaceae family of the order Plantaginales. It was introduced in the middle ages during the Muslim attack. The word "Isabgol" is derived from; "Isap" and "ghol" which is the two Persian words meaning horse's ear because its seed resembles the ear of a horse.

The husk is the most important component of Isabgol which is obtained from its seed. It contains colloidal mucilage consist of Xylose, Arabinose, Galacturonic acid. Its seed contains amino acids including Valine, Alanine, Glutamic acid, Glycine, Cysteine, Lysine, Leucine and Tyrosine and a mucilage consisting of a mixture of polysaccharides composed mainly of Xylose, Arabinose and Galacturonic acid, Rhamnose and Galactose is also present. The seeds also gave aucubin, the antibacterial principle. The seed coat contains fatty acids mainly Linoleic, Oleic and Palmitic acids in decreasing concentrations. The embryo of Isabgol contains 5% of linoleic acid which prevents anti sclerosis.

Isabgol is mainly known for its mucilaginous husk.'Isabgol husk' is the epidermal layer of the seed, which is hydrophilic and it absorbs water by which it increases by 10 times or more in volume. As a result, it stimulates the peristalsis of the intestine and helps in bowel clearing. In the Indian market it is popular as "Sat Isabgol" and It is also used for the termination of pregnancy, as an anti-diarrhea agent and cervical dilator etc. It is also useful against dysentery and intestinal irritation or inflammation. Isabgol husk is known to lower blood cholesterol levels. Apart from medicinal uses, it is also a very good dietary fiber and exploited by blending in confectionary and junk foods. It is also used in the printing industry, as a binder in tablet, as a substitute for agar-agar, making of gum and jelly, etc. The dehusked seeds - commonly known as 'gola' - are used as cattle and poultry feed. Dry straw after harvesting and threshing is also used as fodder; it is also used in Calico-printing, dyeing and ice cream industries as ice cream stabilizers, confectionery, and cosmetic industries.

Fig (i): Isabgol (*Plantago ovata*) 120 days . (Source Google Images)

AREA AND PRODUCTION:

Plantago is native to Iran, India, and Middle East Countries. They are commonly local to some areas of India and Pakistan. It was the first time brought for cultivation in cities of Pakistan i.e., Lahore and Multan. India is the largest producer of Isabgol and export seeds and husk worth ₹ 25 million annually. Rajasthan produces about 67% Isabgol and the rest is by Gujarat 33%.

Districts of Jaisalmer, Barmer and Jalore where Sanchore, a city in Jalore district is the chief producer which accounts for more than 95% production in the state Total area was 123746 ha and production was 68872 tonnes in the year 2008 to 2009. At present, it is increased and reached 184566 ha and total production of 109941 MT of seeds.

It is mainly cultivated in North Gujarat, Kutch, South-western Rajasthan, whereas Haryana, M.P, and Punjab produce a small quantity of Isabgol. After Gujarat, Rajasthan is the second largest growing state. At present Isabgol is also known as the "Dollar earner crop of North Gujarat".

Its cultivation area is about 50000 ha and the annual production is 50 metric tons.

VARIETIES:

Gujarat Agricultural University released two varieties i.e., Gujarat isabgol-1 and Gujarat isabgol-2. CIMAP (Central Institute of Medicinal and Aromatic Plants), Lucknow also released a mutant variety named Niharika, which is a high-yielding variety. Other varieties are TS-1-10, EC-124345, Haryana Isabgol–5, and Jawahar Isabgol-4.

DISEASES ASSOCIATED:

Several diseases were reported on Isabgol from India and other parts of the world.

Fusarium wilt caused by (*Fusarium oxysporum* Schlecht.) was first reported from Arizona, North America (Russel, 1975) as well as from Haryana, India (Mehta *et al*, 1985).

Chastagner *et al.* (1978) reported damping-off caused due to ***Pythium ultimum***.

Powdery mildew caused by *Leveillula taurica* (Lev.) (Arnaud) and (Gupta and Bharadwaj, 1998)

Leaf blight is due to *Alternaria alteranata* (Fr.) Keissler. (Patel *et al.*, 1984) have been reported from India.

Among this downy mildew is one of the most significant and prevalent disease, which occurs in very severe form and causes extensive damage to the crop which results in a decline of its yield and production.

PATHOGEN ASSOCIATION :

Kapoor and Chowdhury,(1976) observed conidiophores in the infected material, oospores were smooth, pale brown to yellow in color and spherical in shape and measures 20µ to 30µ in diameter. *Peronospora plantaginis* have long slender dichotomously branched sporangiophores.

Sporangia are thin-walled, papillate. Sporangia released zoospores in free water. Encysted zoospore germinates through germ tubes and penetrates the host through stomatal openings through aposporium.

Even though chemicals are effective in controlling the disease, the ever-increasing resistance of pathogens to many fungicides and pesticides causing a hazard to the environment has limited its usage and hence there is a growing awareness for the biological control of diseases. This method could be more effective, convenient and a healthy process of controlling and managing the diseases. Moreover, the husk and seed of Isabgol are the widely consuming part and it should not be treated with chemicals or fungicides as it may leave residue of it, and can create many health issues on human beings.

Biological management of diseases can be done in various ways:

- With the help of Bio control Agents for e.g. VAM, Bacteria and other Microorganisms.

- With the help of many plant parts, plant products and other plant compounds.

VESICULAR ARBUSCULAR MYCORRHIZA:

VAM (vesicular-arbuscular mycorrhiza) was first coined by a German plant pathologist named A.B Frank (1855) which describes a symbiotic association between plant roots and fungi. VAM is a sort of mycorrhiza in which the fungus penetrates the cortical cells of the roots of the vascular plant. VAM with its symbiotic association help plants to capture nutrients like phosphorus, sulfur, nitrogen and other micronutrients from the soil. Its hyphae enter into the plant cells, bearing structures that are balloon-like (vesicles) or dichotomously branching invaginations (arbuscules). VAM was used as an earlier term which is now

replaced by AMF (Arbuscular Mycorrhizal Fungi) because only some endomycorrhiza produces vesicles, but mostly all form arbuscules.

Significance of vam :

VAM increases the uptake of nutrients, it also increases the production of growth-promoting substances which provides tolerance to Drought, Salinity and also regulates Defence mechanisms in many plants. The important function of the VAM is to reduce plant pathogens through the induction of systemic resistance. Research has been shown the reduction in many soil-borne as well as bacterial diseases. Few studies have also shown the potential role of VAM in the protection of plants from pathogens.

Mosse (1973) first reported the beneficial effect of vesicular-arbuscular mycorrhizae (VAM) fungi on plant growth. He stated that VAM are important soil organisms which belong to phylum glomeromycota and form symbiotic association with majority of terrestrial plant species.

According to Verma *et.al*(2019), maximum rate of colonisation was observed in *Solanum melongena* which belongs to the family of Solanaceae and *Allium cepa* which was recorded 93.47%, However minimum rate was recorded in family Cucurbitaceae .i.e., 66.7%. There is an increase pressure of population, degradation of useful micro flora of soil increased day by day by use of many chemical fertilisers, fungicides, pesticides and weedicides etc. This has created a chemo phobia for the scientific community. These types of chemical control need an alternative approach which is preferentially the bio control agents specially VAM, which supresses the plant pathogen.

VAM are generally obligatory biotrophs i.e., they feed on the host for their lives and nutrients. They reproduce asexually by spore production. They also form endomycorrhizal association with the root. They also help their host plant by enhancing the process of uptake of water, minerals and poorly mobile phosphorus ions present in the soil.

BIOLOGICAL CONTROL BY PLANTS:

Biological control is the control of disease by the application of biological agents to a host animal or plant that prevents the development of disease by a pathogen. Biological control was discovered by trial and error and then practiced in agriculture long before the term itself

came into use (Baker and Cook, 1974). One example is the ancient practice of not growing the same crop species in the same field more frequently than every second or third year and so on.

The report of Sharma et al(2013) quotes that production of food grain should increase to 250 million tonnes by the year 2020 in order to meet the needs of the growing population. Beyond good agronomic and horticultural practices, farmers rarely rely on chemical fertilizers and pesticides. However, Biological controls should be integrated with other control measures because different methods are effective at different times varying with weather conditions.

The Use of Biological control in Agriculture is less costly and cheaper than any other methods and provides following advantages:

1. Bio control agents give protection to the crop throughout the crop period.
2. They do not cause toxicity to the plants.
3. Bio control agent can be combined with bio-fertilizers.
4. They are easy to manufacture.
5. It is harmless to human beings and animals (Environmentally safe.).

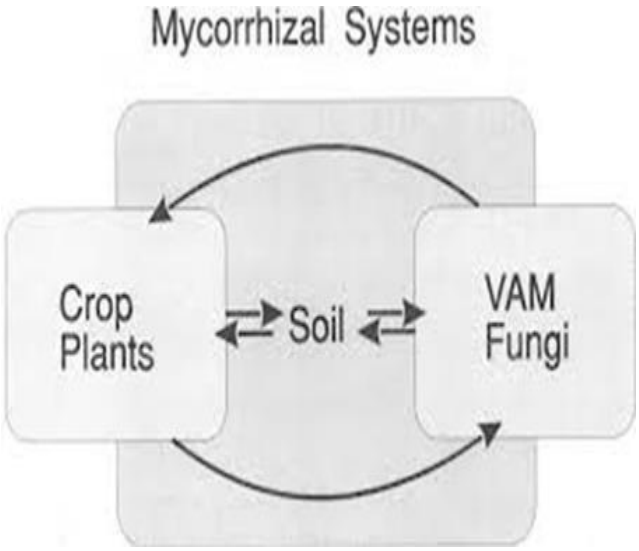


Fig (ii): Showing association between plants , fungi and soil

REVIEW OF LITERATURE:

According to the World Health Organisation**,** 75% to 95% of people in many developing countries rely largely on traditional medicine, mostly herbal remedies, for primary healthcare. In 2006 many researchers estimated that more than 75,000 plant species were being used in medicines worldwide. According to the Botanical Survey of India, India is home of more than 10,000 species of medicinal plants. Our country has a rich history of traditional healing remedies, which later known as Ethno Botany also, many of which list the use of Isabgol (*Plantago ovata*) There is the evidence of the 1670's oldest book named **Hortus Malabaricus** which is a 12-volume book based on the diversity of medicinal plants of the Malabar region along India's west coast.

In many communities, especially indigenous ones, they are still dependent on almost traditional systems of medicine for their health needs, as said by N Anil Kumar (Senior Director and Head of the MS Swaminathan Research Foundation's Community Agro- biodiversity Centre at Kerala's Wayanad) in 2016. Apart from medicinal healing, they have livelihood needs also, such as the medicinal non-timber trees produced by indigenous communities collect and sell for a living, the plants are also a crucial cultural resources.

The latin name of *Plantago ovata* means

1. Plantago – sole of the foot

2. ovate- shape of the seed.

Isabgol suffers with a lot of bacterial and fungal diseases, but Downy mildew is one of the major fungal diseases in Isabgol which is a seed borne as well as soil borne both.

The disease severity was recorded 24.50 to 50.70% and 28.40 to 56.50% in western Rajasthan and 30.70 to 42.30 % and 32.80 to 45.30% in Southern Rajasthan. Severely diseased plants showed a reduction by 28.27 % in seed yield (Yadav and Sharma 2005). *Pernospora plantaginis* is the causal agent for downy mildew in Isabgol. The disease increased with increase in Nitrogen while high dose of Phosphorus reduced the same**.** In this disease systemic infection shows white patches covered with ashy white frost and mycelial growth on the upper and lower surface of the leaf and later turned necrotic with production of oospore. It occurs at the time of spike initiation.

But later on these infected leaves shows curling, crinkling and drying, followed by poor growth of the plant. The infected plants do not produce any seeds which reduce the husk yield by 45 and 20% respectively. External contamination of Isabgol seeds with oospores caused 10.52 and 34.25% downy mildew infection in pots and fields. The plants of middle age (50-60) days were found to be more susceptible compared to younger and older plants
.The estimated annual production is almost 50 metric ton and India earns foreign exchange valued up to Rs.80 crores every year**.**

However not much research has been done on downy mildew of Isabgol whereas other crops related with same disease have proven to control Downy mildew through various methods and practices.

***Peronospora plantaginis* (Causal Organism)**

Peronospora was first described in 1837 by August Carl Joseph Corda, a Czech mycologist and physician, in his first of six volumes *Icones fungorum hucusque cognitorum*. *Pernospora plantaginis* causing downy mildew on Isabgol was first reported by (Desai and Desai 1969). Its asexual stage was studied by (Kapoor and Chowdhury, 1976) on Isabgol.

Majorly it was reported that three different organisms are causing downy mildew of Isabgol. The first reported disease was from Anand, Gujarat. Kapoor and Chowdhury (1976) reported another causal organism *Pernospora alta* Fuckel, later in Rajasthan, Sharma and Pushpendra (1998), observed some new species causing downy mildew was *Pseudopernospora plantaginis* at Udaipur, Rajasthan. Downy mildew pathogens (an Oomycetes) are grouped under Mycota, but in recent advances of the field of fungal systematics it grouped under Oomycetes. Hall (1996) suggested some different methods for its characterisation like protein profiling, sterol and fatty acid profiling etc.

According to Alexopolous, Mims and Blackwell (1996) Classification, *Peronospora plantaginis* classification is as follows:

Phylum : Oomycota

Class : Oomycetes

Order : Pernosporales

Family : Pernosporaceae

Genus : *Peronospora*

Species : *plantaginis*

Pathogen morphology

Desai & Desai (1969) reported that *Peronospora plantaginis* develops white frosty patches on both the lower and upper surface of the leaf which results in poor growth of plant including flowering. In (1976) Kapoor and Chowdhury observed conidiophores as asexual stage in the causal organism, and oospore at sexual stage where oospores were smooth and spherical in shape with a pale brown colour, measuring from 20 to 32 µ in diameter. *P.plantaginis* has long slender and dichotomously branched sporangiophores, some thin walled papillate sporangia were also found.

Peronospora plantaginis produces 2 types of symptoms

1) Systemic: Seedling stage is the initiation of the infection which occurs after 25 days of sowing and lasts throughout the development of Isabgol.

2) Local: This infection mainly occurs on leaf blades as pale brown or bleached spots covered with mycelia growth on lower surface.

P.plantaginis produces approx. 5 to 6 branched conidiophores having 4 to 12 µm branch ends. Though it produces dichotomously branched sporangiophores emerging through stomata.

% Infection in the Crop	Description
i. 0	No disease
ii. 0-20	Small scattered spots on lower leaves
iii. 21-40	Scattered spots on lower and middle leaves and upper leaves free from infection.
iv. 41-60	Lower and middle leaves severely infected all upper leaves with scattered spots.
v. 61-80	All leaves severely infected.
vi. >80	All leaves severely infected and plant ultimately dries and dies.

Table (i)-Effects on the Isabgol plant with respect to the *Peronospora* infection in Percentage (Yadav and Sharma 2005).

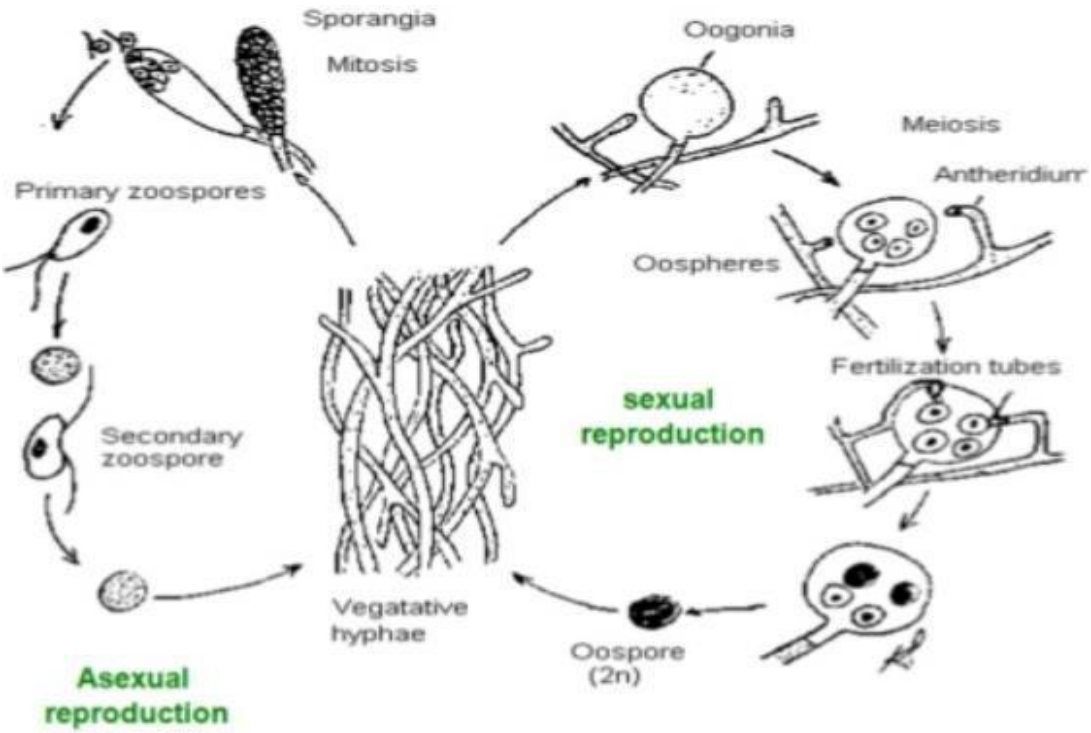


Fig (iii): Life cycle of *Peronospora plantaginis*

BIOLOGICAL CONTROL:

Biological control is the control of disease by the application of biological agents to a host animal or plant that prevents the development of disease by a pathogen. With regard to plant diseases the bio control agents are usually bacterial or fungal strains isolated from the endosphere or rhizosphere. A number of scientists have reported the use of plant parts as bio control agents against various fungal pathogens on plants. Rajeswari and Balasupramani, (2020) reported the *In vitro* evaluation of plant extracts, (garlic, zimmu, neem, tulsi, notchi leaf extract) as bio control agents against leaf blight in pigeonpea.

Management of blight of bell pepper (*Capsicum annuum var. grossum*) caused by *Drechslera bicolor* was also reprted to be controlled by plant extracts of Neem and Tulsi (Jadon *et.al.*2016).

Dheeba *et.al*, (2015) also reported the Efficacy of Neem (*Azadirachta Indica*) and Tulsi (*Ocimum Sanctum*) Leaf Extracts against Early Blight of Tomato.

Invitro Evaluation of Botanicals and Bio control Agents against Pomegranate Bacterial Blight Pathogen was also reported by Patel *et.al* (2015).

Viruses can also be used as bio control agents and there is a resurgent interest in the use of bacterial viruses for control of plant diseases.

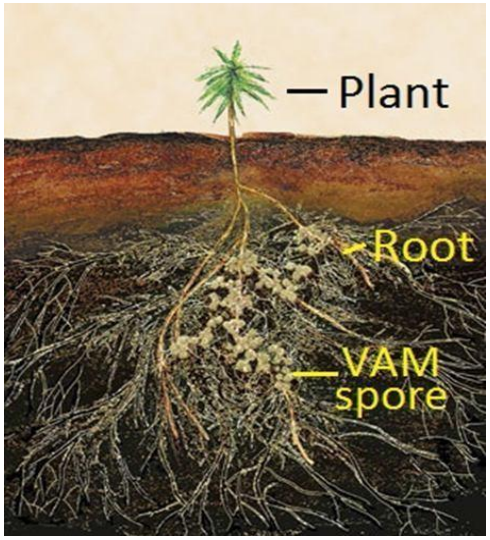
Many successful biological control attempts have also been done on many plants.

- Morphological, molecular and biological evidence reveal two cryptic species in *Mecinus janthinus* Germar (Coleoptera, Curculionidae), a successful biological control agent of *Dalmatian toadflax*, *Linaria dalmatica* (Lamiales, Plantaginaceae)**. (**Roberto Caldara *et.al.*, 2011**)**

- Biological Control and its Important in Agriculture.(Sharma *et.al.*, 2015)

VAM Association in Medicinal Plants:

In an earlier research VAM has been identified in rhizomes. In a survey, some 42 species has been identified mainly from genera *Glomus, Acarlospora, Gigaspora* etc. from the soil associated with medicinal plant species.



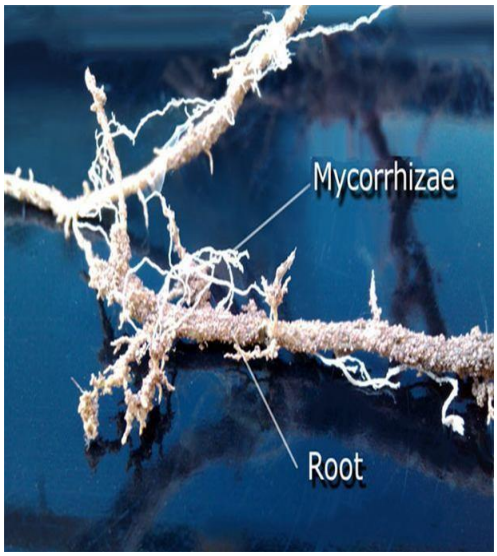


Fig (iv) and (v): VAM species in association with the roots , VAM hyphae in roots.

It is being noticed that VAM influences soil phosphorus content percentage in a greater extent and there will be an increase in the available pool of phosphorus which ultimately decreases the root infection and root colonization percentage in the soil.

VAM associated in Isabgol:

Some of the VAM (vesicular arbuscular fungi) associated with the plant Isabgol are *Glomus fasiculatus , Glomus mosseae , Glomus macrocurpus ,Glomus deserticola* etc. out of which *Glomus deserticola* responded so efficiently in increasing productivity and biomass production. (Mathur *et.al.*2006).

Many of disease control are done worldwide by using agriculture chemicals for controlling downy mildew. But biological control includes the use of live microbial pathogens, some plant parts and its extracts. which are successful in supressing the other population. Biological management of plant diseases can also play an important role in organic and Integrated Pest Management system.

One of the most important things in biological control is to understand about all possible factors causing disease in plants. Some of the factors are environmental moisture and temperature.

Three different pathogens have been reported so far to cause downy mildew of Isabgol. However through all the above information, in this study limitation is with *P.plantaginis* only. Apart from that many attempts for the management of disease has also been made like:

1) **Fungicidal:** Desai and desai (1969); Rathore (1996); Sain and Sharma (1999); Rathore and Pathak (2001) has made many attempts using Aureofungin, Dithane M- 45, Ridomil MZ which were effective against the disease.

2) **Biological** : by *Trichoderma virens* (Patel Nileshkumar,2006) and extracts of Neem and Tulsi, (Patel Nileshkumar ,2006)

Seed treatment with *Trichoderma virens*, *Trichoderma harzianum* and *Pseudomonas fluorescence* significantly increases the seed germination, plant height, size of head, and number of head per plant and root strength. (Shabbir and Mohammad, 2013)

STATEMENT OF THE PROBLEM:

A comparative evaluation of control measures of *Peronospora plantaginis* against Isabgol.

OBJECTIVES OF THE STUDY :

As Isabgol is an important export oriented plant in the market, however downy mildew is one of the major threats for the successful cultivation of plant. Still very less work has been done on this pathogen. The present study was undertaken with an aim to characterize *P.plantaginis* and following objectives were made:

1. To conduct survey, identification and isolation of *Pernospora plantaginis* in the fields of Isabgol in and around Jalore in Rajasthan.

2. To conduct survey, identification and isolation of Vesicular Arbuscular Mycorhizza (VAM) in the soil of Isabgol.

3. To conduct the comparative study between Fungicidal and Biological control.

4. To test the efficacy of the selected Biological control agents against downy mildew pathogen on the growth of *Plantago ovata*.

5. To suggest the best Biological control Agent.

MATERIAL AND METHODS:

a. Location of study

The study will be conducted at the JECRC University, Jaipur (Rajasthan), India during the winter season. However early sowing plant is more susceptible to the disease and can be collected from the field when the plant is of 90 or 100 days. So that the causal organism can be identified at its asexual stage.

b. Plant material used:

Isabgol (*Plantago ovata* Forsk.) will be used for the study. Many other species like Gujarat Isabgol-1, Gujarat Isabgol -2 etc are sown in Western Rajasthan and Gujarat but the cultivar Niharika (HYV) will be used for the study as this variety is grown predominantly in Jalore Region, Rajasthan.

c. Isolation of causal Fungi (*Peronospora plantaginis*):

Diseased leaves will be washed off for the collection of Downy Mildew sporulation by gently brushing the lesions and will be incubated in some humid petri dishes for 12 hours. After that it will be mass cultured on PDA for one week to make a suspension. The drops of spore suspension will be taken on a glass slide with drops of cotton blue + lacto phenol

to count the zoospores. Then the fresh zoosporangia will be suspended into distilled water and will be sprayed with 1 litre capacity with hand sprayer.

Inoculation will be done artificially on different days of the sowing like: after 15, 25.35.45, 60, 80 to 100 days of sowing to find out the highest susceptible stage of the plant for the pathogen response. Record will be taken to note the duration of life cycle of *Perenospora plantaginis* and also to find out the time period of emergence of pathogens asexual stage (More susceptible & responsive on the host plant).

d. Isolation of Vesicular Arbuscular Mycorhizza species:

Since VAM fungi are obligate endo-symbionts and have not been cultured *in vitro*, the only means of identifying these fungi is to collect resting spores from soil and determine the morphological characteristics. By staining roots following appropriate methods, the morphology of vesicles and arbuscules can be understood which also serves as diagnostic characteristic to define spores. Several techniques have been used for the isolation of VAM fungal spores from the soil such as floating technique, gradient centrifugation, monoclonal antibodies and polyclonal antibodies methods, wet sieve process and decanting technique. (Gerdemann and Nicolson, 1963)

The wet sieving and decanting is one of the popular techniques when compared to other techniques. This technique is used for sieving the coarse particles of the soil and retaining AMF spores and organic particles on sieves of different sizes.10 g of soil was mixed with 100ml of water in the 500 ml conical flask. The soil mixture was agitated vigorously to free the AMF spores from soil and allowed to settle for 15-45 minutes and the supernatant was decanted through standard sieves. By using a dissecting microscope, spores were picked by means of pipette or needle.

e. Identification and Study of Vesicular Arbuscular Mycorhizza species :

VAM fungal spores will be studied and calculated under stereomicroscope by seeing its colour, size, spore wall and type of hyphal attachment (Schenck and Perez, 1990). Synoptic keys of Trappe (1982) will be used to study & confirm the genera for further experiments and to maintain the culture.

The Practical set up with pot culture experiments will be done at Botany Lab, JECRC University, Jaipur. Biological trial will be done in following ways:

- Vesicular Arbuscular Mycorhizza will be isolated, identified & mass cultured. The pure and mixed inoculum in different combination of VAM will be added in the pots to measure the control effects on the infected plant.

  VAM will show the antagonistic effect against the spores of *P.plantaginis* present in the soil. The maximum antagonistic effect could be at the asexual stage rather than the sexual stage. This will help in reducing the infection percentage.

- Fresh Extracts of many plant parts can be evaluated against *Peronospora plantaginis* in *in vitro* studies. Fresh and healthy plant parts will be collected and washed thoroughly with tap water, generous anount of each plant part will be macerated in some distilled water (1:1 w/v) by mortar and pestle. Different dilutions of all selected plant and plant parts will be prepared for the antagonistic study against *P.plantaginis* in laboratory.

  Dried Powders of different locally available plant and its parts will be sun dried to make the powder and added (In different concentration & amount) as bio control agents to the soil filled in pots for the bio control experiment setup.

- The experiment will be planned to study the interaction & evaluation of Many fungicides reported to show best results on the downy mildew .Few of them are Aureofungin, Dithane M-45, Ridomil MZ. Combination of these fungicides will be given to the potted plant at different time intervals & different dilutions to find out the minimum standardized concentration/ inoculum of Fungicide vs. biological control agent against *Perenospora plantaginis* pathogen on *Plantago ovata*. The best control strategy and experiment will be recommended.

SIGNIFICANCE OF THE STUDY

Downy mildew is one of the major and wide spread disease experienced by *Plantago ovata* caused by *P.plantaginis*. It causes extensive quantitative as well as qualitative damage to the crop. Use of chemical fungicides as the control strategy are available and massively used by the farmers. But in long run these fungicides are reported to cause gastrointestinal ailments, disorders of the respiratory and reproductive tracts,causing acute and chronic neurotoxicity (insecticides, fungicides, fumigants), lung damage (paraquat), chemical burns (anhydrous ammonia), and infant methemoglobinemia (nitrate in groundwater). A variety of cancers also have been linked to exposure to various pesticides.

In the present study screening and selection of Biological Control Agents will be done which is eco-friendly, causes no side effects on the human health as they are much cheaper than the chemical fungicides. These agents are ready to use, easily available and are economical to the

farmers, which in long run increases its sustainability and develop as a permanent tool to eradicate the pathogen.

SCOPE OF THE STUDY

At present farmers are dependent on the chemically treated fungicides as they are the only option available to the farmers. Hence, they cause many acute and chronic diseases including a number of genetic disorders and cancers. But here we will try to use Biological Agents, which will be eco-friendly, easily available; it reduces the usage of chemical pesticides which creates harmful effects on human health.

LIMITATION OF THE STUDY:

The proposal had following limitations:

1. The experiment is planned with the Isabgol variety/cultivar Niharika only as it is predominantly grown in Rajasthan.

2. Study is focused only on Interaction study of *P.plantaginis* on Isabgol plant. However, other species of *Peronospora* has also been reported. Such as *P.alta* and *Pseudoperonospora plantaginis.*

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